Tissue resident T cell memory or how the magnificent seven are chilling in the bone

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Abstract: Following infection, tissue-resident memory T cells (Trm) are thought to be left behind at sites of antigen encounter to protect affected tissues against subsequent reinfection. In this issue of the European Journal of Immunology, however, Pascutti et al. demonstrate that both murine and human $CD8^+$ Trm specific to seven different pathogens, including systemic, skin, and lung tissue-localized pathogens, accumulate in the bone marrow (BM). These cells have a $CD69^+$ phenotype, develop independently of local antigen, and require IL-15, Blimp-1, and Hobit for their differentiation and maintenance. Following restimulation, these cells expand and rapidly produce cytokines. While some of these responses may protect the BM from infection, the consideration that some of these pathogens or their antigens might never reach the BM suggests additional functional roles of BM Trm, possibly in supporting hematopoietic functions via cytokine production following infection. It will be further interesting to determine whether BM Trm contribute to the circulating effector pool following reinfection with tissue-localized or systemic pathogens and whether these cells can be elicited by vaccination.

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Tissue resident T cell memory or how the magnificent seven are chilling in the bone

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See accompanying article by Pascutti et al.

Abstract

Following infection, tissue resident memory T cells (Trm) are thought to be left behind at sites of antigen encounter to protect affected tissues against subsequent reinfection. In this issue of the *European Journal of Immunology*, however, Pascutti et al. [Eur. J. Immunol. 2019. 49: XXXX-XXXX] demonstrate that both murine and human $CD8^+$ Trm specific to seven different pathogens, including systemic, skin and lung tissue-localized pathogens, accumulate in the bone marrow. These cells have a $CD69^+$ phenotype, develop independently of local antigen and require IL-15, Blimp-1 and Hobit for their differentiation and maintenance. Following restimulation, these cells expand and rapidly produce cytokines. While some of these responses may protect the bone marrow from infection, the consideration that some of these pathogens or their antigens might never reach the bone marrow suggests additional functional roles of bone marrow Trm, possibly in supporting hematopoietic functions via cytokine production following infection. It will be further interesting to determine whether bone marrow Trm contribute to the circulating effector pool following reinfection with tissue-localized or systemic pathogens and whether these cells can be elicited by vaccination.

Until ten years ago, memory T cells were thought to primarily circulate through the body and either home to secondary lymphoid tissues for reactivation (central memory) or to sites of inflammation (effector memory) [1]. It was noted, however, that infection could also induce local depots of memory T cells which remained localized at the sites of pathogen encounter [2], sometimes for years [3]. These non-recirculating cell populations have subsequently been termed tissue-resident memory (Trm) T cells. Both mouse and human $CD4^+$ and $CD8^+$ Trm are characterized by the loss of receptors which allow them to migrate back into the blood stream, including the sphingosine-1-phosphate receptor (S1PR1) which is down-regulated in a Krüppel-like factor 2 (KLF2)-dependent manner due to constitutive expression of CD69 by Trm [4, 5]. In addition, Trm generally express integrins which anchor them in epithelia or collagen within the tissues, including CD103 and CD49a, respectively [6]. The establishment of Trm likely depends on expression of certain chemokine receptors, such as CXCR3 and CXCR6 [7], allowing their initial homing to peripheral sites. In addition, various transcriptional pathways, including NOTCH1/RBPJ, Hobit, Blimp1 and Runx3, have been described for both Trm establishment and maintenance [3, 8]. Depletion of circulating T cell memory cells could demonstrate that murine Trm are sufficient to restrict a variety of infectious disease agents, including viruses, bacteria and fungi [9]. Depletion models were a vital evidence to show that lung Trm provide optimal protection against respiratory syncytial virus (RSV) and influenza A virus (IAV) [10, 11]. Furthermore, $CD4^+$ Trm, which seem to even persist longer in lungs and require less replenishment from the peripheral blood compared to $CD8^+$ Trm [11-13], protect from *Bordella pertussis* and *Mycobacterium tuberculosis* infection in the lung [14, 15]. This protection does not necessarily result from superior effector functions of Trm cells, in which they often resemble effector memory T cells with respect to proliferation, cytokine production and cytotoxicity [16-18]. Instead they seem to be at the right time and the right place to rapidly counter re-infections.

In the study by Pascutti and et al. of the current issue of the *European Journal of Immunology* the authors investigate tissue residency of $CD8^+$ T cells in seven viral and bacterial infections: the herpesviruses Herpes simplex (HSV), human and murine cytomegalovirus (HCMV and MCMV) and the Epstein Barr virus (EBV), influenza A virus (IAV), lymphocytic choriomeningitis virus (LCMV) and the bacterial infection *Listeria monocytogenes* [19]. Despite distinct routes of infection (intranasal, intraperitoneal, intravenous or skin-scarification) and distinct patterns of pathogen localization (systemic, lung-localized, skin-localized) as well as tropism, the authors describe that, in all cases, a pool of pathogen-specific $CD8^+$ Trm is established in the bone marrow (Figure 1). Even when only infection primed $CD8^+$ T cells are transferred to naïve recipient mice, a proportion of these home to the bone marrow and

subsequently express phenotypic and transcriptional hallmarks of Trm, including CD69, CD49a, CD101, and Hobit along with low levels of S1PR1 and KLF2. These murine bone marrow $CD8^+$ Trm were shown to efficiently produce cytokines like IFN-γ, even without restimulation, and to degranulate despite expressing the perforin-mediated cytotoxic machinery only at low levels. These findings provide evidence for the interesting possibility that Trm persist not only at the site of primary infection, but also as a depot in the bone marrow, even despite the absence of cognate antigen or inflammatory cues in the local tissue environment.

The authors further demonstrate that murine bone marrow Trm could be significantly expanded following antigen encounter or infection and, additionally, that sequential infections add to the total Trm pool, rather than “new” Trm replacing the “old” Trm from preceding infections. These findings are both particularly surprising. First, because it has been suggested that the bone marrow mainly maintains resting memory cells [20] and second, because this niche was thought to limit the total memory T cell pool due to the requirement for direct interactions with limited VCAM1 and IL-7-expressing stromal cells [21, 22]. The bone marrow is known to be enriched for memory $CD4^+$ T cells, which accumulate at this site following initial immune responses [21] and can be subsequently mobilized during secondary pathogen encounter to support antibody responses more efficiently than other $CD4^+$ T cell sources [21]. The current study by Pascutti et al, not only suggests that $CD4^+$ memory and $CD8^+$ Trm occupy the same niche within the bone marrow [22], possibly with $CD4^+$ T cells preferentially utilizing IL-7 and $CD8^+$ T cells preferentially utilizing IL-15 as their respective survival cytokines [23], but further, that, unlike $CD4^+$ T cell memory, $CD8^+$ Trm can expand during secondary immune responses at this site without any obvious limitations and may not be mobilized into the periphery at high numbers like $CD4^+$ cells.

What then, is the role for these pathogen-specific bone marrow $CD8^+$ Trm in host protection? The most straightforward possibility is that they restrict subsequent infections at this site. Protection from EBV infection in the bone marrow represents an interesting example. The authors of this study found that EBV specific human $CD8^+$ T cells, despite being present at half the frequency of HCMV specific $CD8^+$ T cells in the bone marrow, were enriched in $CD69^+$ Trm cells. Previously it had been demonstrated that lytic EBV antigen (BZLF1)-specific $CD8^+$ T cells were preferentially enriched in human bone marrow, while latent EBV antigen (EBNA3A)-specific $CD8^+$ T cells were difficult to detect [24]. Lytic EBV replication can be primarily observed in plasma cells in healthy EBV carriers [25]. Additionally, co-infection with the Kaposi sarcoma associated herpesvirus, which stimulates plasma cell differentiation in EBV infected B cells, increases lytic EBV replication [26]. It is known that, following infection, the bone marrow is enriched for plasma cells which are maintained for years due to the survival-promoting tissue niche, providing VCAM-

1, APRIL and BAFF signaling [27]. Thus, immune restriction of lytic EBV infection in bone marrow-localized plasma cells could benefit from a Trm compartment that recognizes early lytic EBV proteins. Furthermore, $CD8^+$ T cell responses against such early lytic EBV antigens have indeed been shown to diminish lytic EBV replication in an in vivo model of EBV infection [28]. Thus, a Trm depot could specifically protect the important hematopoietic compartment from re-infections or chronic infection reactivation in the bone marrow.

Alternatively, the bone marrow may act as a reservoir for $CD8^+$ Trm specific for pathogens, which preferentially infect other tissue sites. In this case, following reinfection, these bone marrow Trm might be mobilized and migrate to the affected tissues to provide protective functions, as has been previously demonstrated for $CD4^+$ memory T cells with a tissue residency profile [21]. Such a scenario would also be interesting from the perspective of vaccination. As vaccines are typically administrated at one site (i.e. the deltoid muscle) to protect from infection at another site (i.e. the respiratory tract), the generation of a bone marrow Trm intermediate which could act as a source for protective memory T cells would be an intriguing possibility.

Pascutti et al., however, raise an interesting additional possible role for these bone marrow-localized $CD8^+$ Trm, namely that the preferential cytokine producing function of these cells, either with or without restimulation, could support steady-state or emergency hematopoiesis [29]. Along these lines it was already shown that IFN-γ by $CD8^+$ T cells promotes myelopoiesis either by stimulating hematopoietic stem cells (HSCs) directly into proliferation [30] or myeloid progenitor cells while retaining HSC quiescence [31, 32]. These functions were suggested to result either from a direct effect of IFN-γ on these hematopoietic stem and progenitor cell populations [30, 31] or via IFN-γ stimulated IL-6 production by bone marrow mesenchymal stem cells [33]. Therefore, infections could elicit a $CD8^+$ Trm cell population in the bone marrow which would support hematopoiesis and stimulate myelopoiesis that would, in turn, support tissue repair following infections. Such a positive feed-back loop for improved hematopoiesis after local and systemic infections via Trm cells in the bone marrow is an attractive theory that would need to be further substantiated in future studies. Nevertheless, the accumulation of $CD8^+$ Trm in the bone marrow after infection, independent of the presence of the causative viruses or bacterium in this tissue, provides a new perspective on the role and functions of T cell tissue residency.

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Conflict of interest

The authors declare no financial or commercial conflict of interest.

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Figure legend

Figure 1: $CD8^+$ T cell tissue residency after lung infection, for example by the influenza A virus. Infections in the lung prime $CD8^+$ T cell responses in the draining lymph nodes. Some of these home into the lung and establish tissue residency after the infection has been cleared. In this issue of the *European Journal of Immunology* Pascutti et al. demonstrate that some of the primed $CD8^+$ T cells also establish tissue residency in the bone marrow and continue IFN-γ production at this site. This figure was created in part with modified Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 unported license: https://smart.servier.com.

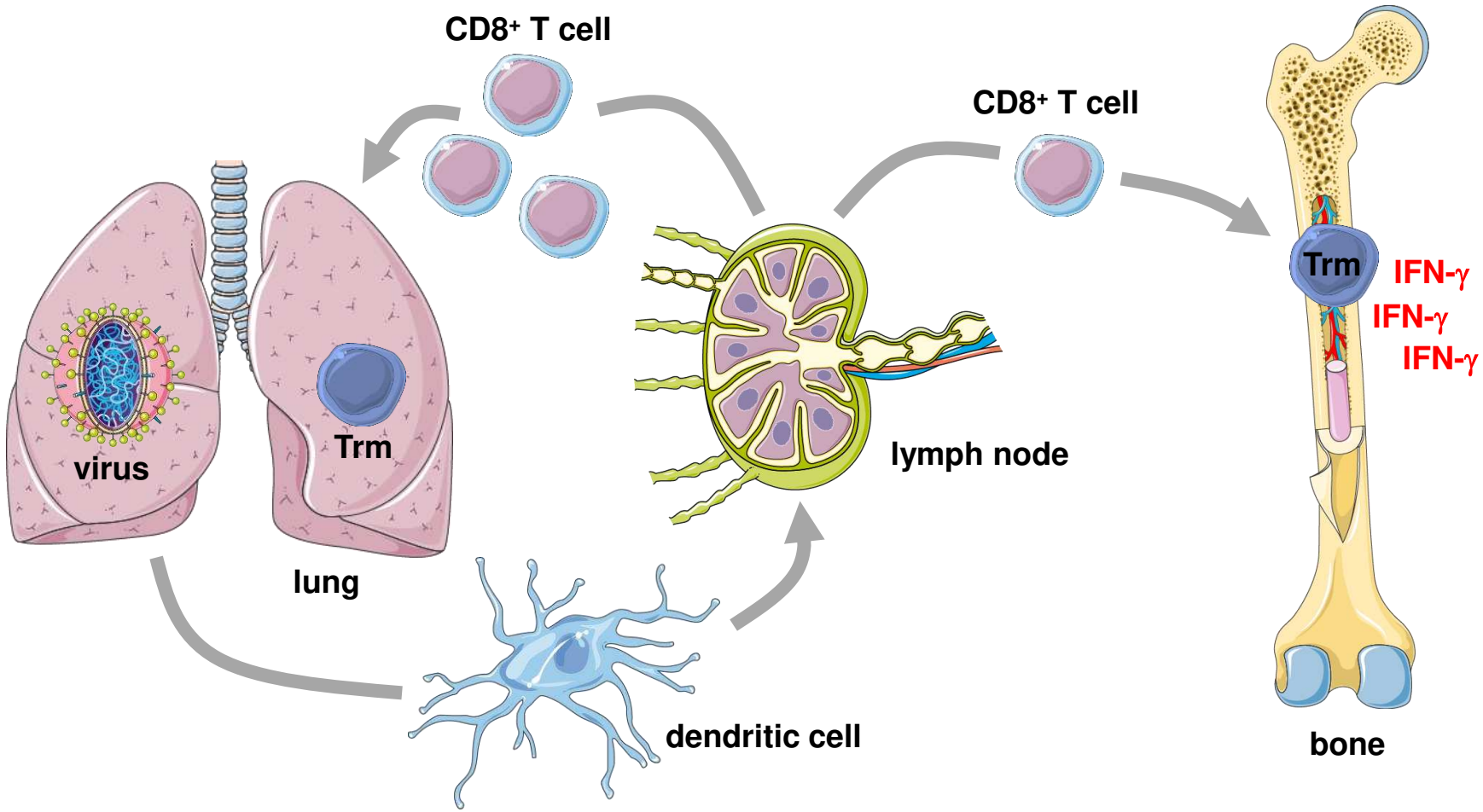
CD8+ T cell
CD8+ T cell
virus
Trm
lung
lymph node
Trm
IFN-γ
IFN-γ
IFN-γ
dendritic cell
bone

Figure 1